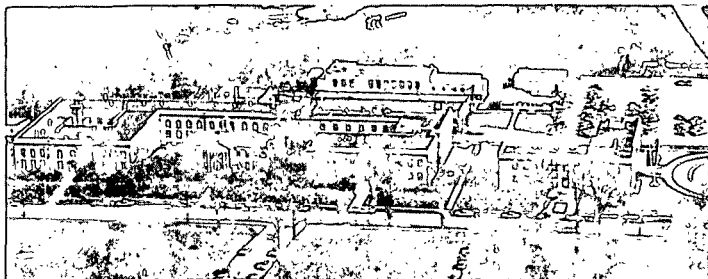


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THE INSTITUTE OF PAPER CHEMISTRY, APPLETON, WISCONSIN

INCREASED YIELDS IN ALKALINE PULPING. I.  
A STUDY OF THE PEELING REACTION AT THE  
CONDITIONS OF KRAFT PULPING

Project 2942

Report One

A Progress Report

to

MEMBERS OF GROUP PROJECT 2942

December 18, 1970

THE INSTITUTE OF PAPER CHEMISTRY

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INCREASED YIELDS IN ALKALINE PULPING. I.  
A STUDY OF THE PEELING REACTION AT THE CONDITIONS  
OF KRAFT PULPING

SUMMARY

An analytical method has been developed for determining the mono- and disaccharides present in the peeling reaction. It involves reduction of the sugars to the respective alcohols, acetylation and determination of the acetates by gas chromatography.

A simple glass reactor has been designed for studying slower kinetic runs at temperatures of 30 to 70°C. Preliminary thoughts are presented for possible flow reactors operating at higher temperatures and under pressure. Such reactors will be used in studying more rapid reactions, with half-lives ranging from two minutes to below a second.

The alkaline systems are terminated by a quenching reaction that is best carried out in two steps: (a) the rapid addition of boric acid to give pH 10, and (b) the slower subsequent addition of sodium borohydride as a reducing agent. The second step is the first step of the analytical method mentioned above. The action of sodium borohydride at room temperature has been found to be most effective at pH 10. At lower pH values reduction is incomplete, due to decomposition of the sodium borohydride. At higher pH values the rate of reduction is slowed down and peeling becomes appreciable as a competitive reaction. Apparently, the sugar is converted into a negatively charged anion which reacts slowly with the borohydride anion.

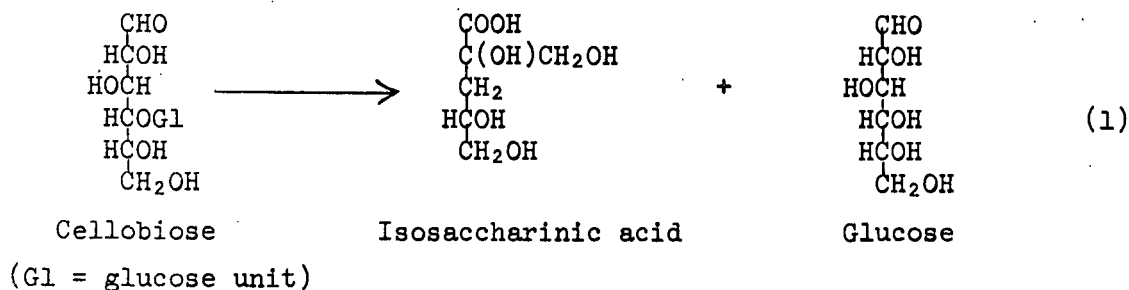
Acetylation of the alcohols is carried out with a mixture of pyridine and acetic anhydride. This reagent is more effective after removal of sodium borate

which is formed in large amounts by the quenching reaction. Treatment with ion exchange resin removes sodium ion and concentration of the acidic effluent with methanol removes the boric acid as a volatile ester.

## INTRODUCTION

The purpose of this project is to study the peeling reaction of oligosaccharides at higher temperatures approaching the conditions used in kraft pulping. This reaction involves the breaking of a glycosidic linkage in aqueous alkali, and is activated by a reducing end group. In the case of polysaccharides, reduced yields obtained in alkali at higher temperatures have been attributed to such a reaction, with the successive peeling off of glucose units as new reducing end groups are formed. Various attempts have been made in the kraft pulping process to block these reducing end groups and thus increase pulp yields. A better knowledge of the fundamental aspects of the peeling process, the goal of this project, will be of great value to a better understanding of alkaline pulping processes.

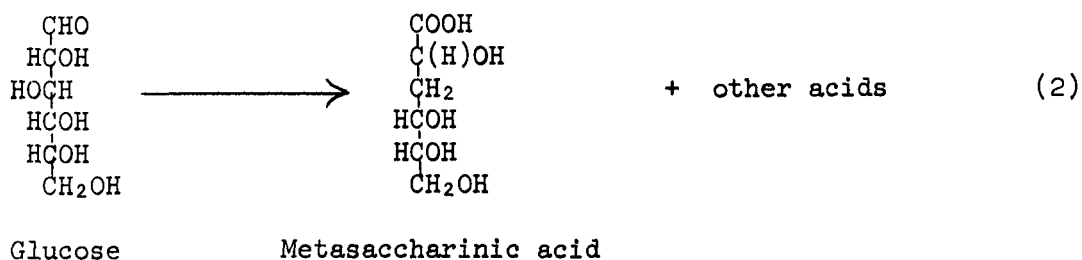
This peeling reaction, as stated in our Proposal, has been studied only briefly and at lower temperatures. The original reaction was first investigated by Corbett and Kenner (1), and involved di-, tri-, and tetrasaccharides. Thus, the reaction of the disaccharide cellobiose with aqueous calcium hydroxide results in the splitting off of the reducing glucose unit as isosaccharinic acid and the formation of glucose from the nonreducing unit, [Equation (1)].



Qualitatively, the reaction can be detected by the formation of the two products, glucose and isosaccharinic acid. In kinetic runs, the rate of disappearance of the disaccharide is determined quantitatively. To a certain extent the rate of formation of organic acidity can be utilized also in kinetic work.\*

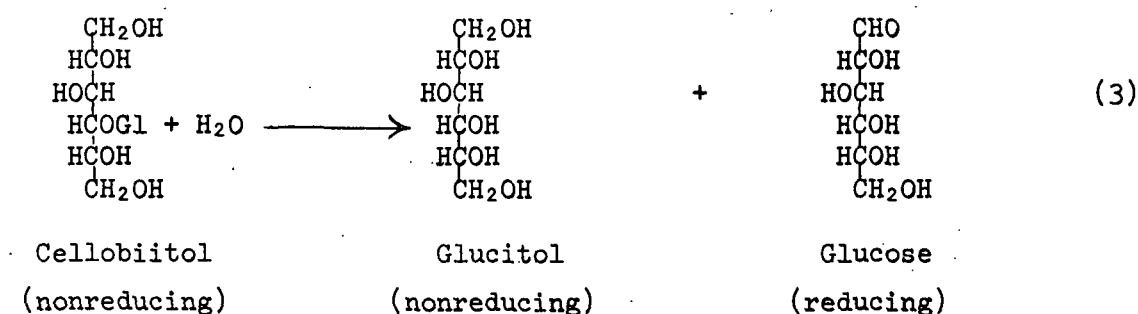
The British workers (1) separated mono- and disaccharides on a carbon column and determined the latter colorimetrically. MacLaurin and Green (2) studied the peeling reaction for cellobiose in 1N sodium hydroxide at 22°C. and separated the various sugars in the system on a borate column. Swedish workers (3) have studied the peeling reaction of cellobiose and two other disaccharides (containing xylose and mannose units) at 60 and 75°C. in dilute (0.2M) sodium and calcium hydroxide. These reactions, faster than those studied earlier, were stopped by addition of boric acid to pH 9; the sugars were then reduced with sodium borohydride. This reduction eliminated the free reducing end groups in both mono- and disaccharides; hence, addition of strong acid hydrolyzed the

\*This development of acidity is not stoichiometric; in addition to isosaccharinic acid formed from the reducing glucose unit, other saccharinic acids are formed by subsequent degradation of the glucose liberated in the reaction. Thus, glucose is an intermediate product and the amount present at any one time is not proportional to the amount of cellobiose removed from the system [Equation (2)].





glycosidic linkages in the resulting "disaccharide alcohols" to give new reducing groups, determined colorimetrically [Equation (3)].



In this way only the disaccharides remaining in the system were determined.

In the present project a modification of the method of Lindberg, et al. (3) has been developed. The C<sub>6</sub> and C<sub>12</sub> sugar alcohols, formed by borohydride reduction, have been analyzed by gas chromatography. This allows not only the determination of the disaccharide remaining but also that of the glucose formed as an intermediate in the peeling reaction.

This analytical method was developed with a thought to the problem of running the alkaline reaction at higher temperatures, where the rate will be great\* and a quick method of stopping the reaction suddenly will be required. The reaction samples will have to be removed from a pressure system, and this may take some time. So an effective way of reducing the pH of the system, and of blocking the reducing end groups that activate the peeling reaction, is a necessary part of an analytical scheme.

\* A rough estimate of the half life of the peeling reaction at 170°C., based on the data at 22°C. (2) and at 60 and 75°C. (3) gives a value of about one second. This estimate is very crude, and is dependent on the strength of alkali in the system.

The study of the peeling reaction at higher temperatures, where the half life will be small (from two minutes to below one second) will require one or more flow reactors, where the following essentials should be observed: (a) rapid mixing of sugar solutions and alkali, (b) maintenance of this reaction solution under pressure at temperatures above 100°C., (c) rapid quenching of the reaction system to stop the peeling, and (d) probable mixing of two solutions at two greatly different temperatures in Steps (a) and (c). Four such types of reactors are discussed in the following pages of this report.

In summary, the project is progressing along two fronts - analysis of kinetic runs at lower temperatures, and planning of flow reactors for use at higher temperatures. The kinetic data obtained at lower temperatures will be extrapolated to give us an idea of possible rates at the higher temperatures, and thus help in the design of suitable reactors.

## STANDARD PROCEDURE FOR SUGAR ANALYSIS

The gas chromatographic method given below is based on the method of Borchardt and Piper (4), used for the analysis of monosaccharides. The original method involved the borohydride reduction of the sugars to alcohols, acetylation of the latter in a mixture of acetic anhydride and sulfuric acid, and separation of the alditol acetates on a column packed with 3% ECNSS-M on Gas Chrom Q 100/120 mesh at 195°C. The present method utilizes acetylation with a mixture of acetic anhydride and pyridine; with this nonacidic reagent, breaking of the glycosidic bond in the disaccharide alcohol cellobiitol is avoided. Secondly, a 5% SE-30 packing on 60/80 acid-washed DMCS Chromosorb W is used for the gas chromatography; this type of column with temperature programming allows faster movement of the alditol acetates through the column and hence obtaining a satisfactory peak for the disaccharide alcohol acetate.

It should be mentioned here that, while the essential peeling reaction is the splitting of the disaccharide cellobiose into glucose and a saccharinic acid, actually in alkali an isomerization of both mono- and disaccharides occur, and so we are dealing with a mixture of three monosaccharides:

glucose, mannose, and fructose,

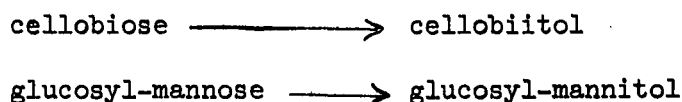
and three disaccharides:

cellobiose, glucosyl-mannose, and cellobiulose (glucosyl-fructose).

When these mixtures of sugars are reduced with borohydride, the respective C<sub>6</sub> or C<sub>12</sub> alditols (sugar alcohols) are obtained (see Fig. 1):

glucose —————> glucitol

mannose —————> mannitol



In contrast, reduction of the ketose isomers, creating asymmetry at C-2, gives a mixture of two alcohols

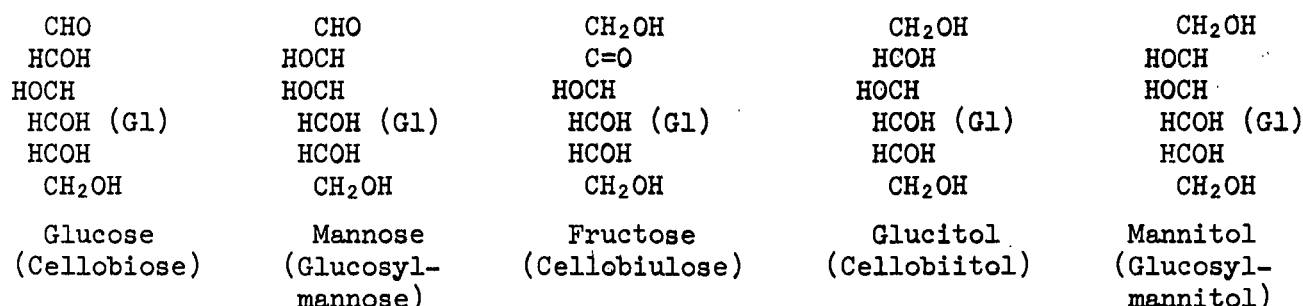
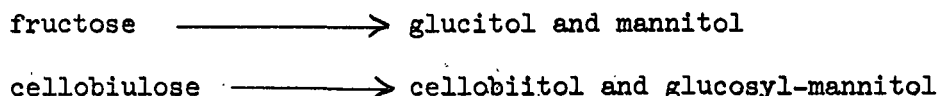


Figure 1. Sugars and the Related Alcohols

Note — The Names in Parentheses are for the Corresponding Disaccharides or Disaccharide Alcohols, With a Glucose Unit Substituted (Gl) at C-4.

In our method of analysis, however, we are concerned only with the relative amounts of mono- and disaccharides, especially the latter, and the gas chromatograph separates the mixtures as such. Thus, both glucitol and mannitol, the two C<sub>6</sub> alcohols, when converted to the acetates, have the same retention time, 6.6 minutes, on the gas chromatograph, and so the area of the given peak obtained is for these two alcohols, or for the mixture of all three monosaccharides in the original reaction system.

Similarly the two C<sub>12</sub> alcohol acetates, those of cellobiitol and of glucosyl-mannose, have the same retention time, about 22 minutes, and so this

"disaccharide peak" represents all three of the disaccharides in the reaction system. This method of analysis, is thus simplified, in contrast to the borate column method used by MacLaurin and Green (2), which determined six separate sugars instead of just mono- and disaccharides.

#### REDUCTION OF SUGARS

A mixture of 50 mg. glucose (or an equivalent amount of other sugars) and 20 mg. cyclohexyl  $\beta$ -D-glucopyranoside (an internal standard) is dissolved in 30 ml. water in a 100-ml. beaker. To this solution is added roughly 200 mg. of sodium borohydride, the beaker is covered with a watch glass, and allowed to stand for 1.5 hours. The solution is stirred briefly with a magnetic stirrer at the beginning of the reduction.

At the end of the 1.5 hours, small quantities of IR-120 resin (washed to above pH 4.5 with good distilled water) are added to the solution with stirring, until hydrogen evolution has ceased. The solution, about pH 4, is filtered and the resin washed with about 3 volumes of water.

The aqueous solution is concentrated on a rotary evaporator at 50-60°C. in a 250 or 500 ml. round-bottom flask. The dry residue will be quite bulky, because of the boric acid present. To this residue is added 100 ml. methanol and the solution concentrated to dryness. The residue, now mostly a sirup, and much less bulky, is again concentrated with 100 ml. of methanol. The sirup, mostly free of boric acid, is dissolved in a little methanol and transferred to a 100-ml. round-bottom flask. If the residue does not dissolve completely, about 5 ml. of water is added, and that transferred, and the large flask washed out with methanol.

The hexitols, glucitol and mannitol, seem to be easily soluble in methanol or ethanol. The disaccharide alcohol, cellobiitol, is soluble in methanol but not in ethanol. (Glucosyl-mannitol, a sirup, has been prepared only in milligram amounts.) The use of water is a precaution to insure total transfer.

#### ACETYLATION OF THE SUGAR ALCOHOLS

The contents of the 100-ml. flask are concentrated to dryness, dissolved in a little absolute ethanol and reconcentrated. The flask is then dried in the oven at 100°C. for 15 minutes, removed and cooled to room temperature. To this flask is added a mixture of 6 ml. pyridine and 5 ml. acetic anhydride. The residue in the flask should all dissolve in this acetylation mixture. The flask is stoppered and left overnight (15 hours or more) at room temperature.

The acetylation solution is then poured into a 100-ml. beaker containing a magnetic stirring bar and about 60 ml. ice. The mixture is stirred slowly until part of the ice has melted. The cloudy solution is poured into the flask to rinse out any acetylation solution and poured back into the beaker. The solution is stirred until the ice has melted.

The water solution is then extracted in a 250-ml. separatory funnel with 3 20-ml. portions of chloroform. Each extract is run into another 250-ml. funnel\* mounted on a ring stand below the first funnel. The combined extracts are then washed with: 60 ml. 1N HCl, 0.1N HCl, and 3 x 60 ml. distilled water. The extract is run into a 125-ml. Erlenmeyer flask, dried over sodium sulfate, filtered from

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\*The extractions can be done quite rapidly with the two funnels, if the chloroform extract, after each washing is run into the other funnel. The first funnel is then rinsed with water, and used to collect the extract after the next washing.

the latter, and the filtrate concentrated at 50°C. in a 100-ml. flask to dryness. The dried extract is then dissolved in 5 ml. acetone for gas chromatography.

#### GAS CHROMATOGRAPHY

An appropriate amount of the acetone solution (1-5  $\lambda$ ) is injected into the gas chromatograph. The instrument is a Varian Aerograph Model 1200-1, Hy-Fi III; hydrogen flame ionization detector. The column is a 5 ft. x 1/8 in. SS, 5% SE-30 on 60/80 acid-washed DMCS Chromosorb W. Suitable analyses result with either on-column or off-column injection. The carrier gas was prepurified nitrogen (30 p.s.i.g. off-column injection, 35 p.s.i.g. on-column injection; this could vary between machines). Hydrogen was used at 10 p.s.i.g.; this gave the maximum response. The column temperature was programmed from 175 to 275°C. at 4°/min. and hold. The injector and detector temperatures were both 300-310°C.

With the above temperature program the approximate retention times of the several alcohol acetates were as follows:

Glucitol or mannitol acetate - 6.6 min.

Cyclohexyl glucoside acetate - 10.5 min.

Cellobiitol acetate or glucosyl-mannitol acetate - 22 min.

In contrast, glucose pentaacetate ( $\alpha$  or  $\beta$ ) was about 5.4 min., and cellobiose octaacetate 21 minutes. The lower retention time of glucose pentaacetate allows a detection of peeling in the alkaline reduction of cellobiitol.

The retention time of  $\alpha$ -isosaccharin acetate is about 2.3 min. on the above temperature program. This is the acetylated lactone, and represents the main saccharinic acid formed in the peeling process [see Equation (1)].

An example of such a recording with several peaks is shown in Fig. 2. The areas of the peaks are calculated from the tracings on the disk integrator on the recorder, allowing for deviations from a standard base line. From these areas are calculated the response factors for the given compounds in relation to the internal standard.

The response factor,  $F_{i/x}$ , is defined by the formula given below, and in the present work is related, for the several acetylated alcohols, to cyclohexyl  $\beta$ -D-glucoside as an internal standard.

$$F_{i/x} = (A_i M_x) / (A_x M_i)$$

where  $A_i$  = the integral of the internal standard peak in arbitrary units

$A_x$  = the integral of the unknown peak

$M_i$  = moles or molar concentration of the internal standard

$M_x$  = moles or molar concentration of the unknown

For each sample at least three chromatograms are run. For these triplicate analyses the areas are measured and the response factor calculated in each case. The three factors are averaged to a final average response factor. In the following tables where only response factors are given, each such factor represents three separate injections of a given solution into the gas chromatograph, the measurement of the resulting areas for the chromatogram, calculating to obtain a factor, and averaging to obtain the final factor.

The advantage of an internal standard is that it is stable in the alkaline system, and relative changes in the given sugars can be related to it and



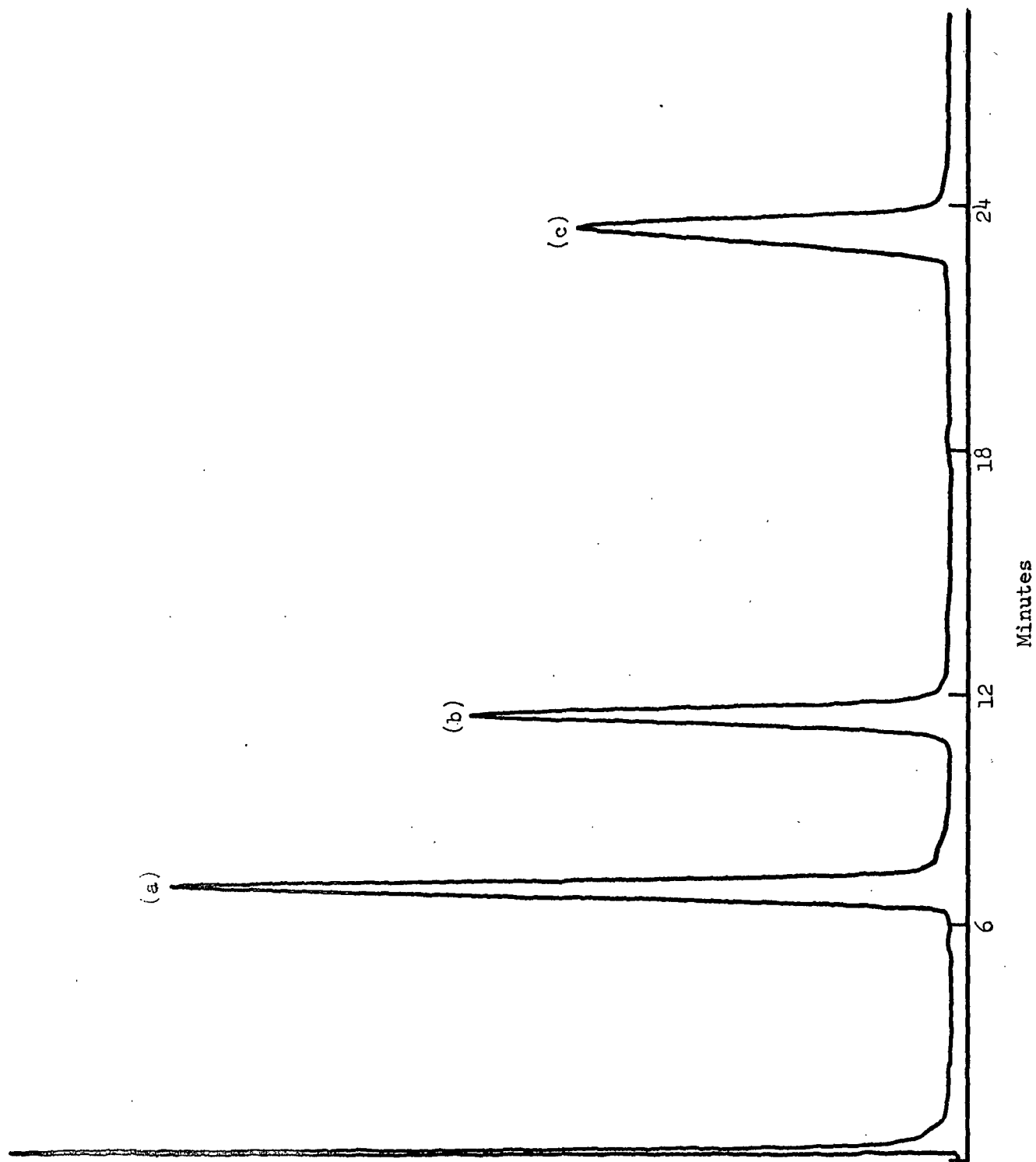


Figure 2. GLC Recording Showing Peaks for (a) Glucitol, (b) Cyclohexyl Glucoside, and (c) Cellobiitol, all as Acetates. The Peak on the Left is the Solvent

converted to absolute values. Thus, only an approximate aliquot of the original reaction solution, when converted to the sugar alcohol acetates, need be injected on the gas chromatograph. The concentration of the internal standard in the original solution is known, and so the relative amounts of sugars in the aliquot are related to this concentration.

#### RESPONSE FACTORS FOR THE SUGAR ALCOHOL ACETATES

These are given in Table I, and in relation to the internal standard, cyclohexyl  $\beta$ -D-glucopyranoside. This standard was used, as its retention time is between that for the two hexitol acetates and for cellobiitol acetate. In the earlier method of Borchardt and Piper (4), inositol acetate was used for a standard, but with the present column used (SE-30) the retention time for this compound is the same as for the hexitol acetates.

A less polar column is used in this work than was used by Borchardt and Piper; this was necessary in order to handle the disaccharide alcohol, cellobiitol, and to use a higher temperature program, up to 275°C. The earlier work on the monosaccharides used a maximum column temperature of only 195°C.

It will be seen that the response factors and retention times for glucitol and mannitol are similar; therefore, a mixture of these two alcohols can be analyzed as one peak in kinetic studies. Such a mixture will be formed by the reduction of fructose, or by the alkaline isomerization of glucose formed in the peeling reaction. Presumably there is the same similarity of response factors for cellobiitol and glucosyl-mannitol acetates; the latter compound has not been reported in the literature and has been prepared for this project as a sirup by the borohydride reduction of 20 mg. of glucosyl-mannose (2) and subsequent acetylation.

TABLE I  
RESPONSE FACTORS OF KNOWN POLYOL ACETATES IN RELATION  
TO THE INTERNAL STANDARD

<u>Glucitol Response Factor</u>			
No.	$\frac{M_x}{M_i}$	$\frac{A_i}{A_x}$	$\frac{F_i}{x}$
26-1	0.4892	2.704	1.323
		2.709	1.325
		2.745	<u>1.343</u>
			1.330
26-2	0.2545	5.260	1.339
		5.078	1.292
		5.117	<u>1.302</u>
			1.311
Av.			1.321
<u>Mannitol Response Factor</u>			
27-1	0.4909	2.753	1.351
		2.743	1.346
		2.707	<u>1.329</u>
			1.342
27-2	0.2472	5.368	1.327
		5.613	1.388
		5.204	<u>1.286</u>
			1.333
Av.			1.338
<u>Cellobiitol Response Factor</u>			
27-2	0.3004	2.611	0.7845
		2.574	0.7732
		2.666	<u>0.8010</u>
			0.7862
28	0.3401	2.367	0.8049
		2.295	0.7805
		2.232	<u>0.7590</u>
			0.7815
Av.			0.7839

## ACETYLATION WITH AN ACIDIC REAGENT

The earlier methods of sugar analysis involving only monosaccharides used a mixture of acetic anhydride and sulfuric acid for acetylation of the sugar alcohols. In the present work the disaccharide alcohol, cellobiitol, contains a glycosidic bond and this is susceptible to hydrolysis or acetolysis in an acidic reagent.

An experiment showed such acetolysis. Cellobiitol (50 mg.) was acetylated one hour at 50-60°C. in 7 ml. of 15/1 v/v acetic anhydride-sulfuric acid (1). The solution was then cooled, and poured onto ice and water. The chloroform extract was washed with water, and concentrated to a sirup. The latter was analyzed by gas chromatography and showed two small peaks for glucose acetate and glucitol acetate. The area of the two small peaks is about 3% of that of the large cellobiitol peak.

## SELECTION OF PYRIDINE-ACETIC ANHYDRIDE AS A SUITABLE REAGENT

Two nonacidic acetylation reagents were explored. The first was a mixture of acetic anhydride and pyridine (5:6 v/v) employed for 15 hours at room temperature. The other was a mixture of 5 ml. acetic anhydride and 100 mg. sodium acetate, employed for one hour at 80-100°C. The first reagent gives a clear solution with the given alcohols. The second reagent initially gives a mixture of solid and liquid; after a few minutes heating the alcohols dissolve completely, but some sodium acetate remains even at 100°C. Magnetic stirring is used with this latter reagent.

Cooling with ice is necessary when water is added to the pyridine-acetic anhydride solution, as the water dissolves readily in this reagent. With the

sodium acetate-acetic anhydride reagent, however, water dissolves only slowly in the acetic anhydride (or vice versa) and so heat evolution is small.

Two series of experiments were carried out to judge the effectiveness of these two methods of acetylation. The first series, given in Table II, comprises the acetylation of mixtures of two hexitols, mannitol and glucitol, in the two types of reagents. The second series, given in Table III, are for the reduction of a mixture of glucose and cellobiose, in the presence of an internal standard, and then acetylation of the resulting glucitol and cellobiitol without isolation; again two methods of acetylation were employed. Also, in this second series, the trimethylsilyl ethers were also prepared, but found unsatisfactory; two peaks were obtained in the case of cellobiitol. A noisy base line was also found.

TABLE II

ACETYLATION OF KNOWN HEXITOLS WITH TWO DIFFERENT ACETYLATION REAGENTS

Expt.	<u>Composition of Original Sample</u>			Method of Acetylation	$\frac{F_i}{x}$
	Glucitol	Mannitol	CHG		
23-1	9.78	--	11.57	Sodium acetate	1.269
23-2	9.78	--	11.57	Pyridine	1.335
24-1	--	10.13	9.79	Sodium acetate	1.290
24-2	--	10.13	9.79	Pyridine	1.294

Note - Composition of sample is given in mg.; response factor is for the given sugar alcohol present. CHG = cyclohexyl  $\beta$ -glucoside.

TABLE III  
REDUCTION OF SUGARS AND DERIVATIZATION OF THE RESULTING  
ALCOHOLS, WITHOUT ISOLATION

Original Reactants		Weight, mg.	Mole x 10 <sup>4</sup>
Cellobiose		45.6	1.332
Glucose		57.8	3.208
Cyclohexyl $\beta$ -glucoside		41.2	1.577
Sodium borohydride		200	52.9

Expt.	Derivatives	$\frac{F_i}{x}$ , glucitol	$\frac{F_i}{x}$ , cellobiitol
18A	Acetate (sodium acetate method)	1.113	0.737
18B	Acetate (pyridine method)	1.245	0.706
18C	Trimethylsilyl ether	0.8948	0.605

Note - Retention times for TMS derivatives of glucitol, cellobiitol, and cyclohexyl  $\beta$ -glucoside were approximately 5.1, 18.1, and 19.0 (two peaks) and 10.1 minutes. The original reduction was carried out in 50-75 ml. water for 2.0 hr. at room temperature.

It was concluded that the pyridine-acetic anhydride reagent was the most suitable one to use, from the viewpoint of response factors, and ease of application in the laboratory (soluble system and lack of heating).

A comparison of the response factors in Tables II and III is given in Table IV; also given are the response factors for the pure crystalline alcohol acetates, originally given in Table I and considered to be the "ideal" factors. It can be seen in Table IV that the pyridine method of acetylation gives the better response factors, starting with the hexitols. When the variable of borohydride

reduction (starting with the sugars in 18A and 18B) is introduced, the pyridine method is still better than the sodium acetate method for glucose. With cellobiose the sodium acetate method seems to be slightly better than the pyridine method.

TABLE IV

COMPARISON OF RESPONSE FACTORS FOR ALCOHOLS ACETATES  
OBTAINED BY TWO METHODS OF ACETYLATION

Expt.	Starting Material	Method	Glucitol	$\frac{F_i}{x}$ for Mannitol	Cellobiitol
26,27, 28	Known crystalline acetates	none needed	1.321	1.338	0.7839
23-1, 23-2	Known hexitols	sodium acetate	1.269	1.290	
23-2, 24-2	Known hexitols	pyridine	1.335	1.294	
18A	Glucose and cellobiose	sodium acetate	1.113		0.737
18B	Same	pyridine	1.245		0.706

Note — The above data are compiled from Tables I-III.

The overall picture, derived from the data in Table IV, is that pyridine is the better acetylating agent. Now an attempt will be made to improve the reduction step, and to obtain precision, so that a reproducible calibration curve can be obtained for the several sugars.

One unknown variable is the stability of the internal standard. It has been assumed that cyclohexyl  $\beta$ -D-glucoside is stable in the borohydride reduction step and that the acetylation step is satisfactory. This will be checked in future work, using glucitol as an internal standard in the reduction step.

An example of a satisfactory analysis is given in Fig. 3, where the use of the response factor for glucitol acetate from Table I gives three values very close to 100% for original glucose present.

#### EFFECT OF BORATE ON ACETYLATION

Two sets of experiments were carried out with an alkaline acetylation reagent, sodium acetate-acetic anhydride. In the first set (Experiments 13 and 14 in Table V), mannose was reduced with sodium borohydride but in one case the excess borohydride was decomposed by addition of acetic acid, and in the other case IR-120 resin was used. The two aqueous solutions were concentrated to dryness and then reconcentrated with methanol to remove boric acid. However, only in the second case (Experiment 14) was this successful. In the first experiment (Experiment 13) a bulky residue was obtained after methanol concentration.

TABLE V  
EFFECT OF BORATE ON ACETYLATION

Expt.	Starting Compound	Borate Present	GLC Peaks, retention times in minutes
13	Mannose	yes	7.5 and 8.4
14	Mannose	no	8.4
15	Mannitol	no	8.7
16	Mannitol	yes	7.2 and 8.4

The subsequent acetylations with sodium acetate-acetic anhydride show the adverse effect of borate ion. When borate ion is present, two peaks are obtained on the gas chromatograph, but only one when it is absent.



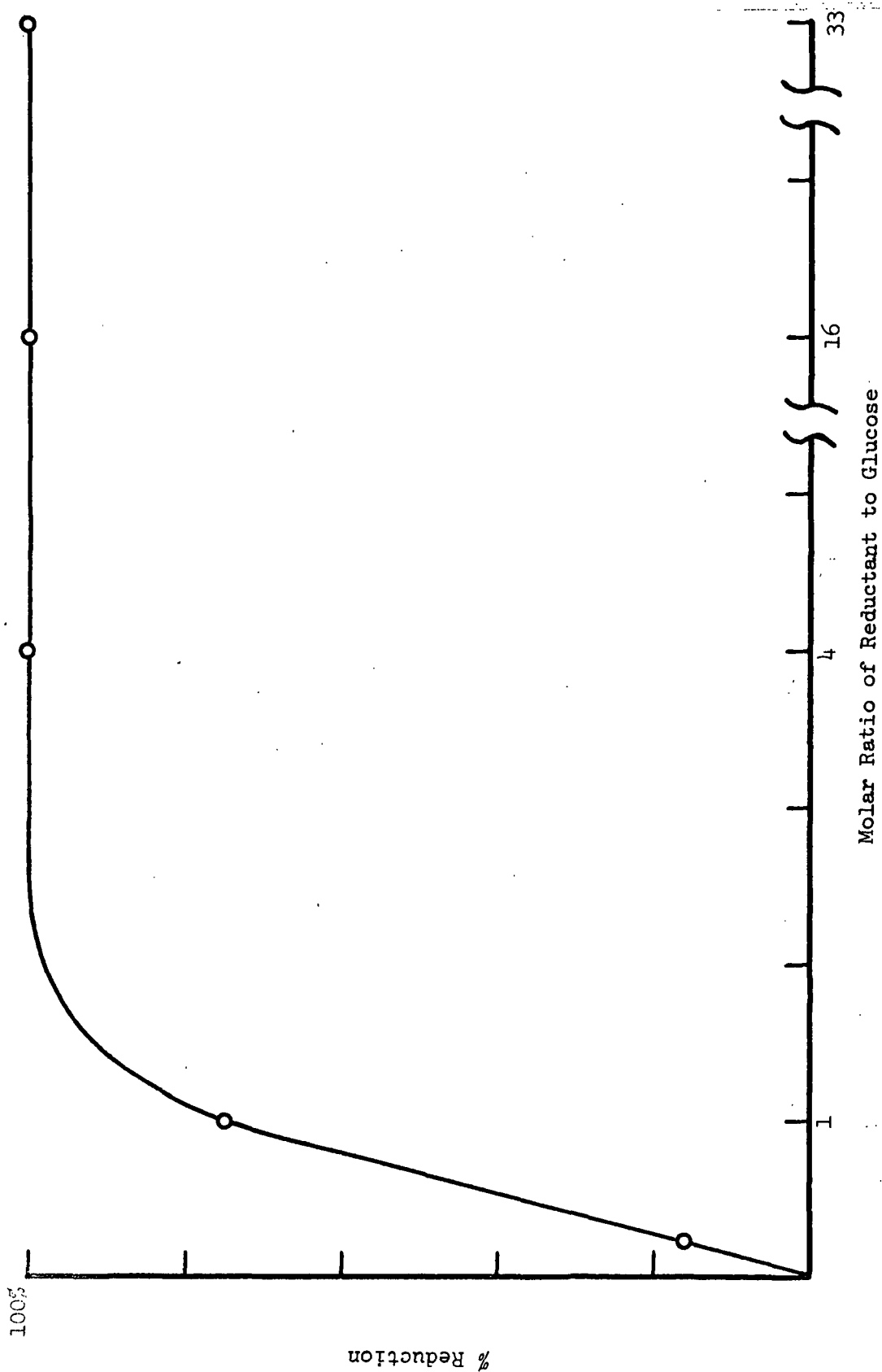


Figure 3. Effect of Excess Sodium Borohydride on Reduction of Glucose

Similar results were obtained when mannitol was the starting material; this was treated with sodium borohydride, and again both acetic acid and IR-120 resin were used. The subsequent gas chromatography shows two peaks when borate ion is present, and one when it is absent.

In earlier analyses for pentoses and hexoses, acetic acid has been used to decompose excess borohydride (1); the borate present is not removed and the subsequent acetylation with acetic anhydride-sulfuric acid is not affected. However, in basic acetylation systems the borate is able to complex and affects the acetylation.

When acetic acid is used to decompose sodium borohydride, a mixture of sodium acetate and boric acid is formed. Such a mixture will not form methyl borate when treated with methanol, as the alkaline sodium acetate will saponify the weak ester. In contrast, use of IR-120 resin removes sodium ion and leaves only boric acid. The aqueous solution in this case, on concentration, will give a solid residue of boric acid (and sugar alcohols) which will readily react with methanol to form the volatile methyl borate. Hence, boric acid can be easily removed from such a system by concentration with methanol.

The data in Table V are for acetylations with sodium acetate-acetic anhydride. It is assumed that similar data would be obtained with pyridine-acetic anhydride, and with either glucose or cellobiose as the starting material.

#### EFFECT OF EXCESS BOROHYDRIDE ON THE REDUCTION OF SUGARS

Sodium borohydride has a low molecular weight (38) and four active hydrogens. Theoretically, 38 g. of this reducing agent should reduce 720 g. (4 moles) of glucose. However, an excess of reagent is normally needed to drive

a reaction to completion and a very dilute solution of sodium borohydride, with a pH below 10, tends to decompose. So the 1/18 ratio of reductant to sugar is impractical.

In Fig. 3 a graph is shown for varying amounts of reducing agent per gram of sugar, ranging from 0.25 to 33.4. It can be readily seen that a 4:1 ratio on a weight basis is sufficient, or about 200 mg. sodium borohydride to 50 mg. sugar in 30 ml. water. This 4:1 ratio is about the amount used by other workers in the analysis of simple sugars.

#### CONCLUSION ABOUT THE ANALYSIS OF SUGARS BY THE ABOVE METHOD

The method seems to be satisfactory, with most of the adverse variables ironed out. Now it is a matter of obtaining reproducible data for subsequent kinetic work.

## PREPARATION OF REFERENCE COMPOUNDS

The preparation of several acetylated derivatives is given below. These compounds were used in the determination of retention times and response factors in the gas chromatographic analytical method. The first two compounds are not new, but cellobiitol nonaacetate has not been reported in the literature. It was obtained as a sirup at first but crystallized after a month's standing. The last two compounds were prepared on a small scale, and are sirups.

## GLUCITOL HEXAACETATE

This was prepared by heating 10 g. of glucitol (sorbitol), 5 g. of anhydrous sodium acetate, and 100 ml. of acetic anhydride with stirring to 105°C. on a hot plate. Most of the solid material dissolved and only a small amount of fine residue remained. The solution was allowed to cool to 45° (1.5 hr. total reaction time) and, as a thick paste, was poured onto 200 ml. of ice. The mixture was stirred one hour, the solid precipitate filtered, washed with water, and recrystallized from 50 ml. 95% ethanol. The product melted at 99-101°C. (corrected).

## MANNITOL HEXAACETATE

This was prepared similarly. The product melted at 124-125°C. (corrected).

## CELLOBIITOL NONAACETATE

This compound was originally prepared from 0.5 g. cellobiitol, by heating with 0.2 g. sodium acetate and 5 ml. acetic anhydride. The original product, after addition to water, was a sirup and was extracted with chloroform. This sirup finally crystallized, and could then be readily crystallized from either absolute

ethanol or an ether-cyclohexane mixture. The crystals melted at 104-106°C. (corrected). This compound has not been previously reported in the literature.

Since cellobiitol is not readily available, a preparation was made starting with 1.0 g. cellobiose, reducing with 1.00 g. sodium borohydride, treating with IR-120 resin, and then after concentration with methanol to remove boric acid, acetylated with sodium acetate and acetic anhydride. The cellobiitol, before acetylation, was a clear sirup, soluble in methanol but insoluble in ethanol. The product, after crystallization from ether-hexane, gave two crops; 0.840 g. m. 103-105°C. and 0.550 g. m. 100-102°C.

A larger reduction, starting with 10 g. of cellobiose, gave only 6 g. of acetate initially, and a sirupy mother liquor, which was gradually recrystallized from ether-petroleum ether. Finally a pure crop, 2.7 g. m. 103-105°C., and a second crop, 3.1 g. m. 103-104°C., were obtained. Other crops, with lower melting points were obtained.

#### GLUCOSYL-MANNITOL NONAACETATE

This acetate was prepared as a sirup by starting with 20 mg. of glucosyl-mannose (2), reducing with sodium borohydride and then treating the reaction mixture with IR-120 resin, methanol and finally pyridine-acetic anhydride as in the standard procedure for sugar analysis.

#### $\alpha$ -ISOSACCHARIN ACETATE

This acetate was prepared as a sirup by acetylating 100 mg. of crystalline  $\alpha$ -isosaccharin in 15 ml. 3:1 v/v pyridine acetic anhydride overnight. The solution was poured into water, and chloroform extraction gave a sirup.

## THE REACTION SYSTEM TO BE OBSERVED IN THE PEELING REACTION

A kinetic study of the peeling reaction consists primarily of mixing two aqueous solutions, one containing a sugar and the other alkali, allowing the reaction solution to react for a given length of time at a given temperature, and then stopping the reaction by either lowering the pH or blocking the active end groups or both. For a reaction that is relatively slow this is just a matter of mixing solutions in small glass containers in a thermostat and taking samples at appropriate times. However, when the reaction is much faster, with a half-time of only a few minutes, or into the second or millisecond range, manipulations such as mixing of solutions, etc., have to be much faster and cannot be done manually.

A survey of the literature concerning fast reactions shows that it is confined to two areas (a) that of low temperatures, i.e., below 40°C., and (b) that most of the work has been done in the biological field. Apparently, no work has been done with fast chemical reactions at high temperatures under pressure. Correspondence with Dr. Octave Levenspiel of Oregon State University has not been fruitful; he has written a book on Chemical Reaction Engineering but knows of no work in the high pressure area for very short time intervals.

We have corresponded with Dr. Britton Chance of the University of Pennsylvania who has been one of the leaders in fast biological reactions; he feels that his apparatus is capable of working at high pressures and this equipment is discussed below. We have also corresponded with Dr. Edward M. Eyring at the University of Utah; he has always worked at room temperature and referred us to Dr. Robert L. Berger of the National Institute of Health. Some of Dr. Berger's work is cited also.

The literature survey in the following section is given to present ideas that have been applied to follow the kinetics of fast reactions, although at mild conditions. It is hoped that some of these ideas can be successfully applied to our present problem. Also it is felt that more correspondence or even a visit to Dr. Chance would be fruitful. A great amount of material for this review has been taken from a book on fast biochemical reactions that was edited by Dr. Chance and others (5).

A brief outline of possible procedures during kinetic runs is given below, before the literature review of flow reactors, so that the reader will be able to relate our immediate problem with the techniques that have been used in studying fast biological reactions.

Alkaline reactions will be studied at temperatures up to 170°C. This necessitates the following mixing reactions, and heating and cooling (quenching) involving aqueous NaOH (up to 2 Normal) and aqueous sugar solutions (cellobiose and glucose).

1. Mixing a large volume (25-ml. basis) of alkali and a small volume of carbohydrate (1 ml.). Two items are involved here.
  - a. Using hot alkali (at 170°C.) and a cold solution of carbohydrate. At a 25:1 ratio, the large amount of hot alkali should give a resultant high temperature for the mixture and avoid the problem of thermal decomposition for the carbohydrate in aqueous solution alone.
  - b. The problem of mixing the two solutions rapidly. Further in this report are given various designs for multijet and other (coaxial) types of mixers. Most of these mixers, used in biological work, are made of lucite, which will not be resistant to hot alkali.
2. Carrying out the alkaline reaction for a definite period of time. This will involve a certain dwell time in a length of tubing, in milliseconds at the shortest. The biologists have studied such systems but only at lower temperatures (37°C.).

3. Quenching or stopping the reaction. This can be done in two ways, or actually a combination of these two methods.
  - a. Cooling the reaction mixture by addition to a solution at room temperature or lower. (The biologists have used cryogenic methods, down to very low temperatures, but they are studying reactions at only  $37^{\circ}\text{C}$ ., and in our case, room temperature is  $150^{\circ}$  cooler than  $170^{\circ}\text{C}$ .) This will involve a mixing chamber again and a relatively large volume (150 to 300-ml. basis) of cold solution.
  - b. Reduction of pH. This is best done with 0.5M boric acid, which will give a buffer region of pH 10 very rapidly. For 2N NaOH, 4 volumes of boric acid will be required, and the cooling effect of such a large volume ratio will be great. Thus, 1 volume at  $170^{\circ}\text{C}$ . and 4 volumes at  $0^{\circ}\text{C}$ . will give a resultant temperature of about  $35^{\circ}\text{C}$ . Such a quenched solution will be relatively stable.
  - c. A third step, following reduction of pH, is the immediate addition of sodium borohydride to convert the reducing sugars to alcohols. This reduction is the first step of the analytical scheme, and it is felt advantageous to apply this reaction here.



## A LITERATURE REVIEW OF FLOW REACTORS

### SYRINGE REACTORS

The original flow reactor was designed by Hartridge and Roughton (6) and consisted of two pistons or syringes pushing reactants into a mixing chamber, as shown in Fig. 4. The reaction solution was then passed through an observation tube where it was analyzed continuously by optical methods. A more elegant modification was the stopped flow method; the apparatus of Gibson (7) is shown in Fig. 5. The flow of liquid through the observation tube pushes a float P that seals open Q and stops the flow of reactants from the two syringes. The movement of a vane ahead of float P is timed electrically and this movement related to optical measurements made on the solution at O. Thus, the time of analysis of the solution can be measured in milliseconds.

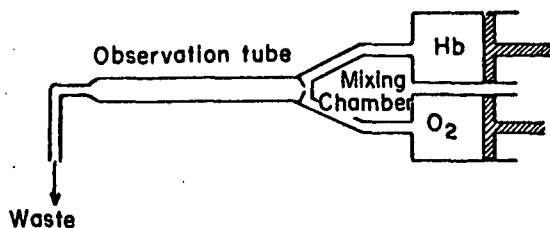


Figure 4. Hartridge and Roughton Method  
Ref. (6)

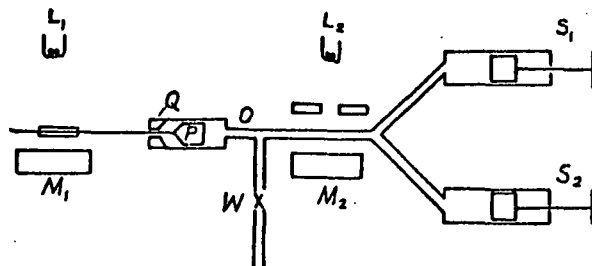


Figure 5. Stopped Flow Method  
Ref. (7)

Most of the biological reactions have been measured continuously by optical methods and the stopped method used frequently. In our work, however, samples will have to be removed from the alkaline system and quenched to allow a more leisurely analysis of the sugars present. The following section cites some of the sampling methods that have been employed in biological work.

#### SAMPLING METHODS

A rotating stopcock with an evacuated bore is shown in Fig. 6. This method, discussed by Longberg-Holm (8) allows rapid sampling at 1.5-sec. intervals; the sample is flushed from the evacuated bore with water into a sample tube. Presumably, this could be used with an alkaline system, but (a) glass cannot be used with hot alkali, and (b) the flushing solution will be at a much lower temperature than the reaction solution. Mixing hot and cold liquids might cause problems.

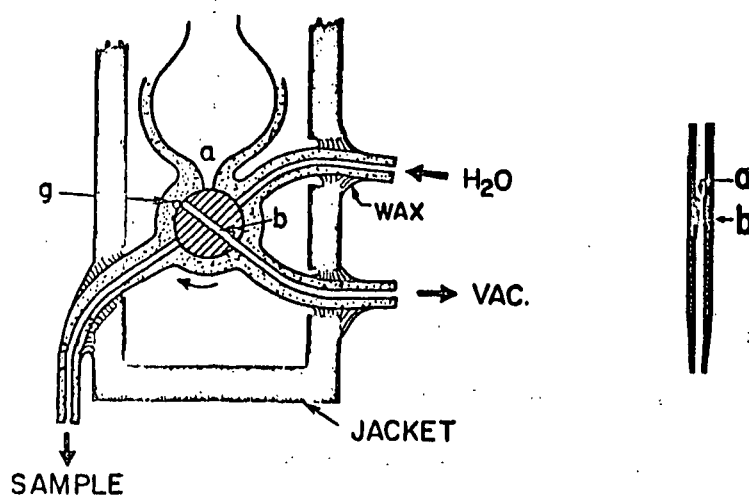


Figure 6. Stopcock Sampler Ref. (8)

A more sophisticated aspirator sampler, shown in Fig. 7, has also been discussed by Longberg-Holm (8). When the aspirator tube is pushed down, the side opening in the tube enters the reaction solution, and a stream of water through the tube sucks some solution from the reaction vessel and empties it into a sample tube below. By precise electrical timing, with a solenoid and a return spring on the aspirator tube, samples can be taken at short time intervals.

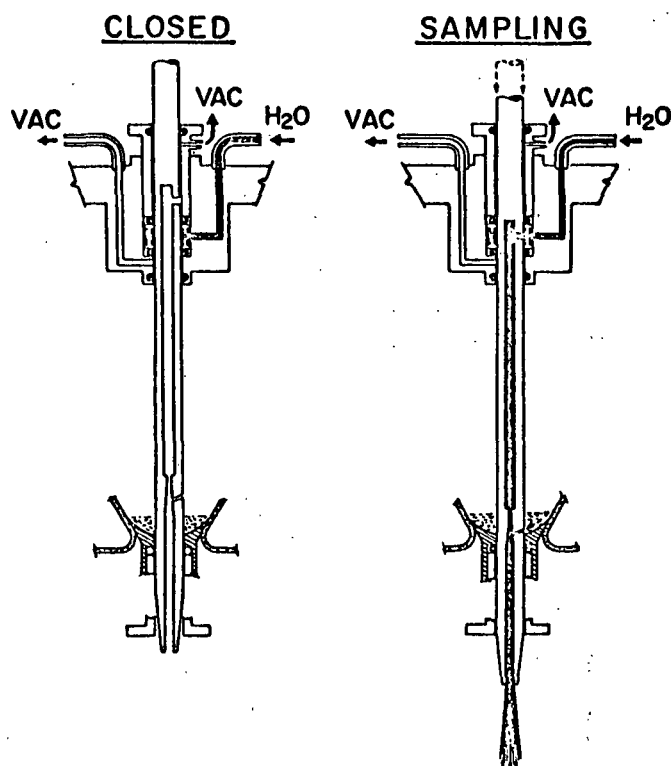


Figure 7. Aspirator Sampler Ref. (8)

Miettinen (9) has designed an automatic fraction collector (Fig. 8) that will take samples down to a reaction time of 0.2 sec. This apparatus might be useful for studying reactions in the interval between a half-time of five minutes and that of a few seconds, operating at atmospheric pressure.

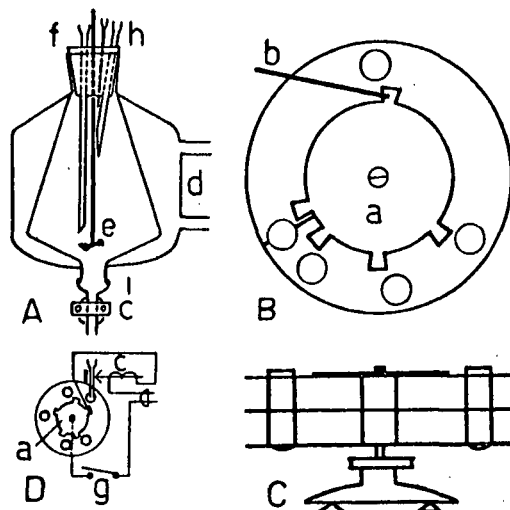


Figure 8. Rotating Automatic Sample Collector Ref. (9)

- A = Reaction Vessel Containing the Cell Suspension
- B = Sample Collector, Top View
- C = Sample Collector, Side View
- D = Switching Diagram

#### QUENCHING METHODS

In most of the biological work the reactions have been monitored continuously by optical methods and only rarely have samples been taken for subsequent analysis. These samples have to be quenched to slow the reaction down rapidly and this involves addition of the reaction solution to a second solution that inactivates the given biological system. Thus, in addition to mixing two solutions initially to create a reaction, a second mixing step occurs to stop the reaction.

A syringe reactor with capillary exits was employed by Barman and Guttfreund (10). The equipment was a modified version of Gibson's topped flow method (see Fig. 5); the stopping syringe P was removed and replaced with capillary tubes (1-mm. diameter) of varying lengths, feeding into quenching solution.

The reaction time was determined by the length of the capillary tube. The authors were able to obtain satisfactory quenching by just simple injection from the capillary, without stirring the quenching solution. They even found stirring tended to slow the quenching, due to vortex formation. They suggested preventing this vortex effect by using smaller jets at the end of the capillary tube or else using ultrasonic treatments.

The authors used this reactor to study the alkaline hydrolysis of 2,4-dinitrophenyl acetate. The saponification was carried out in 0.2 to 0.5N NaOH and the 3-ml. samples taken were quenched with 4N HCl, then adjusted to pH 4 (addition of 2M KOAc) and the optical density read for liberated phenol. The first-order plot obtained is shown in Fig. 9. The authors were interested in this plot only as an evaluation of their reactor for use in certain biological reactions.

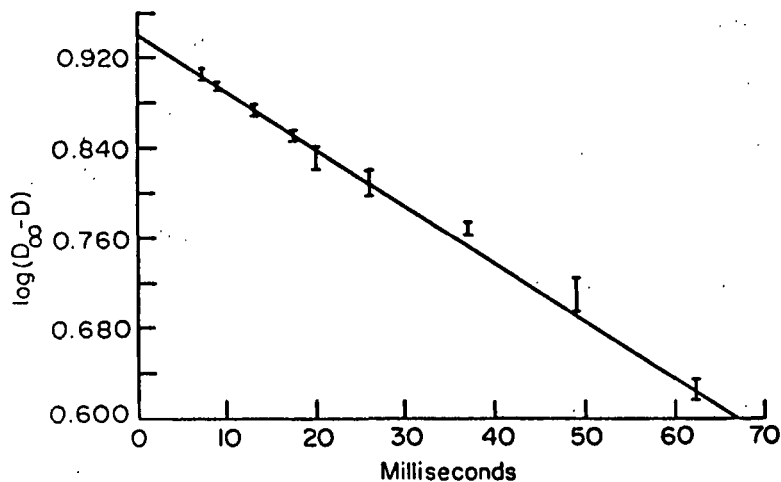


Figure 9. First-Order Plot of Alkaline Hydrolysis of Dinitrophenyl Acetate Ref. (10)

A 3-syringe injector, designed by Miettinen (9) is shown in Fig. 10. The three chambers contain the two reactants and a quenching solution, and the first two are mixed in bulb e before they encounter the quenching solution in bulb f. This is a very simple and supposedly reliable apparatus, and reaction times down to 2 milliseconds can be obtained. This is a batchwise device and each reaction point has to be obtained separately. The sample size is limited to 1-2 ml. volume. The bulbs are of simple construction and there is no mention of using jet-mixers to obtain rapid mixing of the three solutions involved.

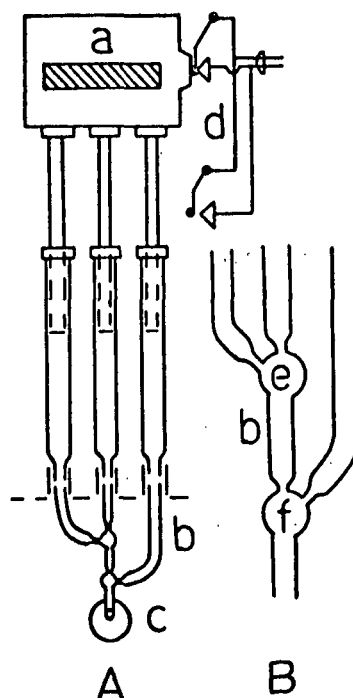


Figure 10. 3-Syringe Injector for Short Reaction Times. A: Entire Apparatus, B: Capillary Part Enlarged Ref. (9)

Pinsent (11) has designed a flow reactor, with special emphasis on the quenching aspects (Fig. 11). It consists of 4 syringes whose plungers are driven by a common pushing device, P. Syringes A and B contain reactants that are mixed

at X. X is a two-stage mixer; each stage consists of 4 jets, mounted tangentially so that a swirling action occurs. Solution A enters the first stage, with a clockwise swirl and Solution B enters the second stage (1 mm. farther along the tube between X and Y) with a counterclockwise swirl. The opposed swirls give efficient mixing and greatly reduce the pressure needed to drive the solutions.

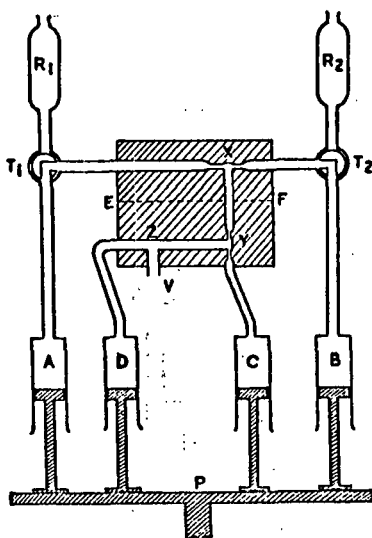


Figure 11. Design of Quenching Apparatus  
(not to Scale) Ref. (11)

The quenching solution, C, is added at Y, which again is also a two-stage mixer, similar to that at X. The quenched solution is then passed to the T-joint at Z where a second reagent (not a quenching agent) is added; this reagent, D, is added to facilitate subsequent analysis and speed of mixing is not necessary here.

The four syringes are of 5 to 10-ml. capacity. The tubes XY and YZ are of 3-mm. diameter. Results were obtained with  $\pm 7\%$  precision for a reaction with a half-life of 400 milliseconds. The geometry of XY can be adjusted for reactions of 50 milliseconds half-life to 2 seconds or longer. R<sub>1</sub> and R<sub>2</sub> are reservoirs to fill A and B; similar reservoirs for C and D are not shown, to allow a simpler sketch.

## MIXERS USED IN FLOW REACTORS

When very short reaction times are used, the need of rapid mixing is important, and workers, beginning with Roughton, have designed multijet mixers, where a series of small holes or jets, set at a slight angle, introduce the given solution into a central chamber; a swirling action is set up by the tangential direction of the jets. One mixer designed by Chance and Legallis (12) is shown in Fig. 12, and another by Berger (13) in Fig. 13. Berger describes his device in detail in his chapter in Chance's book and also the original Hartridge-Roughton apparatus. On page 35 he mentions the problem of mixing high and low viscosity solutions. This may be the case of  $2N$  NaOH and dilute sugar solutions. There is no mention of mixing two solutions at different temperatures, such as we have proposed.

It should be kept in mind that for the mixing of two reactants that two such mixers are often used, one to introduce each reactant. Czerlinski (14) has designed a curved coaxial mixer with an infinite number of jets, shown in Fig. 14. This may be easier to construct of steel than the preceding mixers, which were often made of plastic. In the coaxial mixer the two reactants are introduced at A and B, respectively. The type of flow is shown in Fig. 15 and is discussed by Berger (15).

A very simple type of mixer is the ball mixer, shown in Fig. 16 and discussed by Berger and Bowman (16). In such a mixer there is turbulent flow behind the sphere.



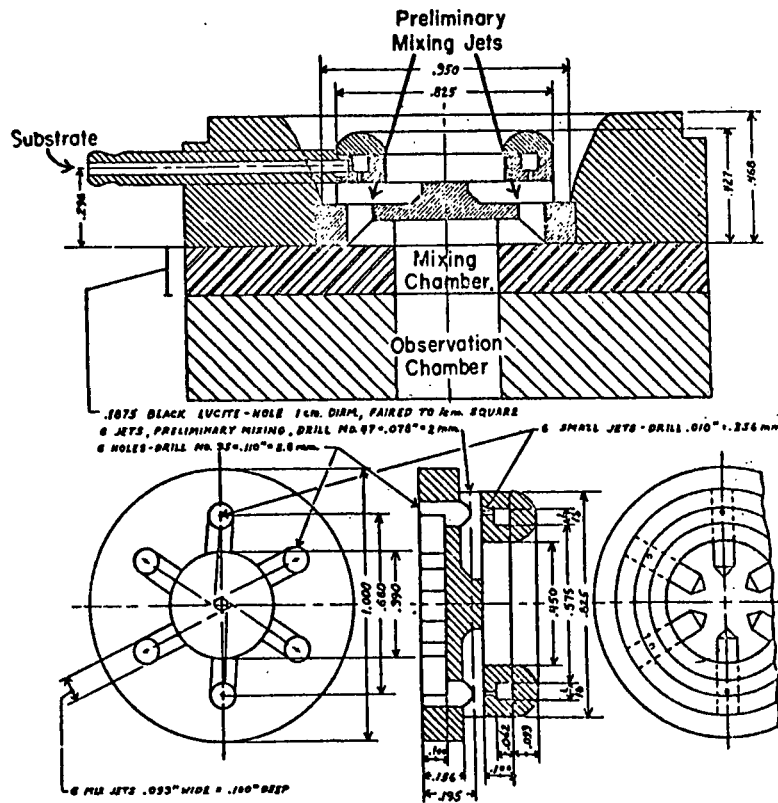


Figure 12. Multijet Mixer Ref. (12)

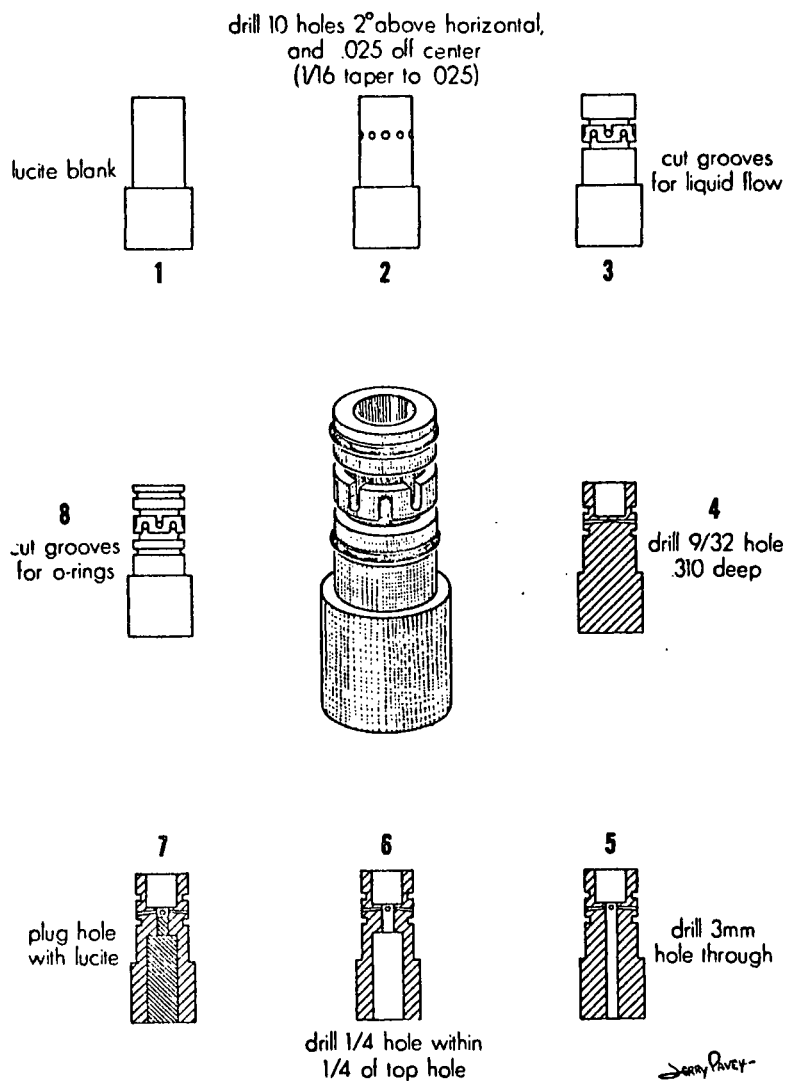


Figure 13. Ten-jet Mixer Ref. (13)

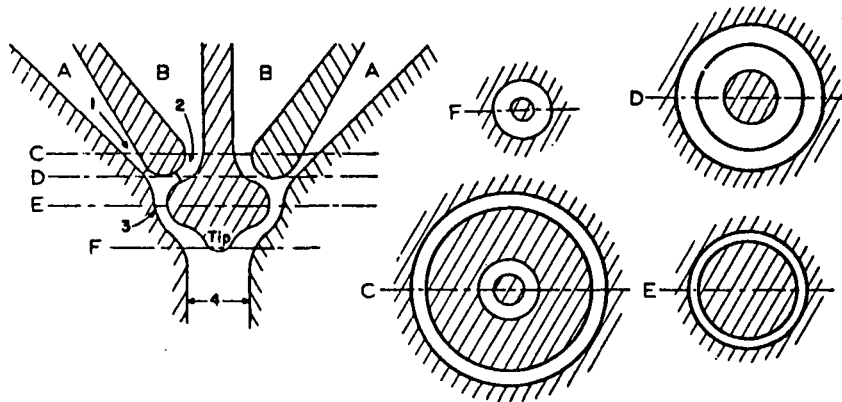


Figure 14. "Infinite Jet Mixer" for Solutions A and B. On the Right, 4 Cross Sections Through the Planes C, D, E, and F. At the Left, an Axial Cross Section of the System Ref. (14)

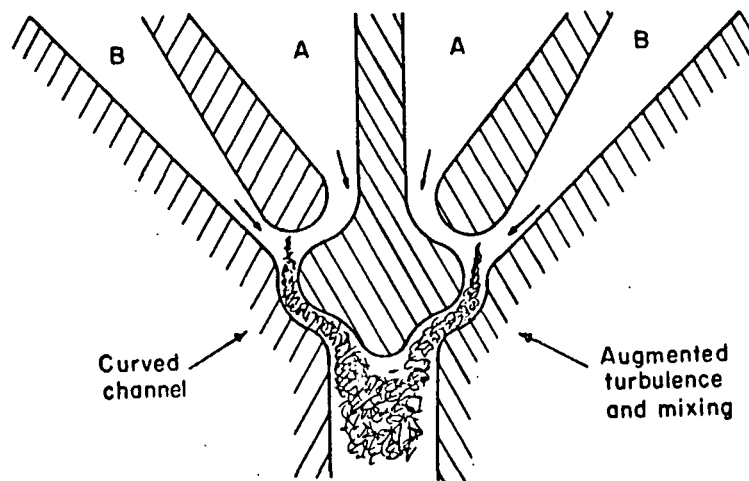


Figure 15. Postulated Mixing Ref. (15)

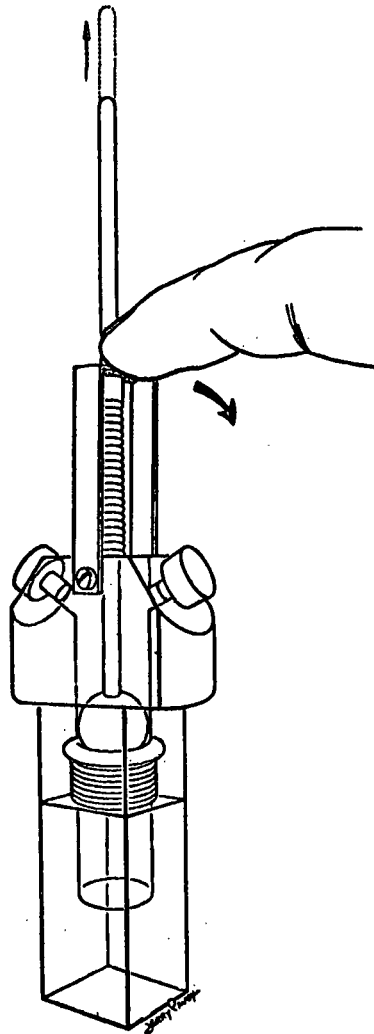


Figure 16. Cuvette Ball Mixer  
Ref. (16)

## PRESSURIZED FLOW REACTORS

Normally the syringes for the reactors described above are moved by a pushing device, often a weight actuated by a solenoid and a spring return. However, in two cases, at least, gas pressure is used to move the syringes, and this pneumatic action allows very fast movement but also means that the respective syringes must be fitted with gas-tight O-rings, etc.

Czerlinski (14) used nitrogen tanks, shown in Fig. 17, to drive two concentric syringes. At driving pressures of 50 atm., flow speeds of about 70 m./sec. were obtained. The base of the compound syringe F is held in an open position by nitrogen pressure in the two tanks E. The two solutions are introduced at valves A and B. When a solenoid G is tripped, a valve below the base of F is opened, and nitrogen pressure is introduced from the left tank E. The syringe F is then driven upward so that the two solutions are mixed below H and the resulting reaction solution is analyzed optically at H. The velocity of the rapidly moving mixture is measured at J. The rapid movement of the syringe F is finally stopped by the "saucer springs" at K.

The second apparatus designed by Chance and Legallis (12) is shown in Fig. 18. This consists of a large and a small syringe in the upper part of the apparatus, containing substrate and enzyme solutions, respectively. These two syringes are driven by a common pushing block so that the two solutions are mixed and observed in an observation chamber (optically) as they pass into a lower syringe. While this apparatus was operated with 20 atm. air, giving 600 lb. force on the syringe pistons (17), it has been tested at static pressures of 65 atm. The time of flow studied was about 100 milliseconds until the lower syringe hit the stop, so that very short intervals of time were observed.

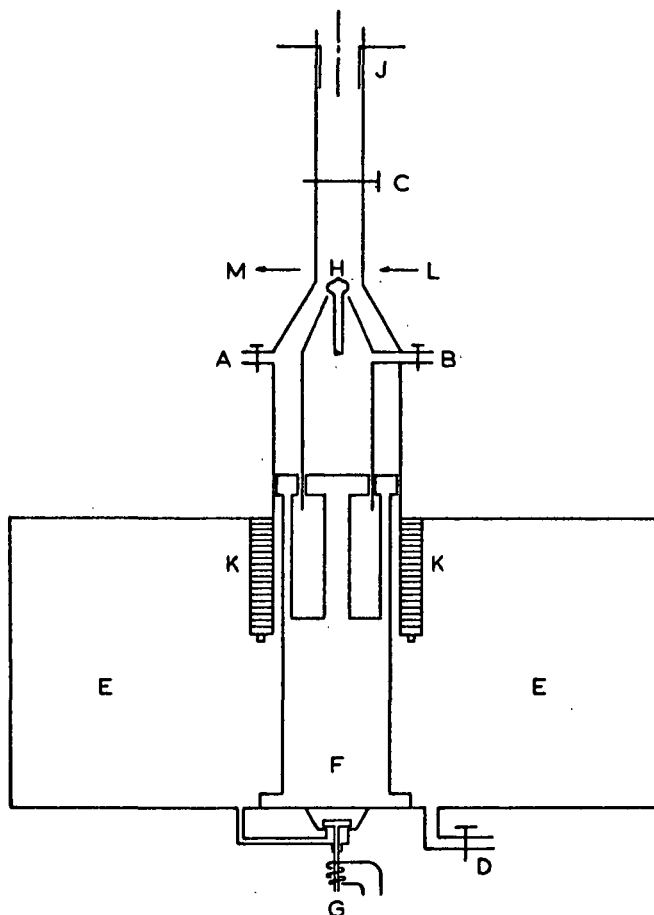


Figure 17. Concentric Syringe Reactor  
with Pneumatic Drive Ref. (14)

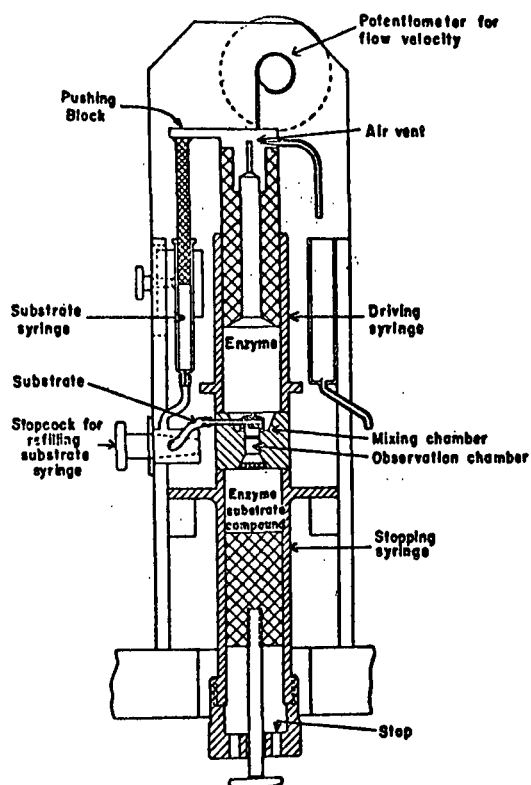


Figure 18. Pneumatic Reactor of Chance  
and Legallis Ref. (12)  
(A = Interior View)

### POSSIBLE REACTORS FOR THE PEELING REACTION

Kinetic studies of the peeling reaction will encompass three areas:

- (a) Relatively slow reactions at 30 to 70°C. that can be handled in simple apparatus manually; the half-lives of such reactions will be above 5 minutes, so that samples can be taken readily at certain time intervals.
- (b) Faster reactions, of half-lives ranging from 5 minutes down to a few seconds, and carried out at temperatures ranging from 75 to 90°C. Samples will have to be taken at shorter time intervals by some sort of mechanical device or flow system, and the higher temperatures will not allow manual handling.
- (c) Very fast reactions, of half-lives in the range of seconds to milliseconds, and carried out at temperatures above 100°, up to 170°C. so that pressures up to 10 atm. will be encountered. Such equipment will have to be carefully designed to take samples at very short time intervals and extreme care will be needed in efficient mixing of the several solutions involved.

### SLOW REACTIONS AT ATMOSPHERIC PRESSURE

The simple glass tube, shown in Fig. 19, has already been used in trial kinetic runs at 30°C. The outer part of the tube contains 25 ml. of 2N sodium hydroxide and the inner tube, sealed to the bottom of the outer tube, contains 1 ml. of carbohydrate solution. The tube, after flushing with nitrogen, is sealed with a simple screw cap, and immersed in a thermostat for 30 minutes to allow the two



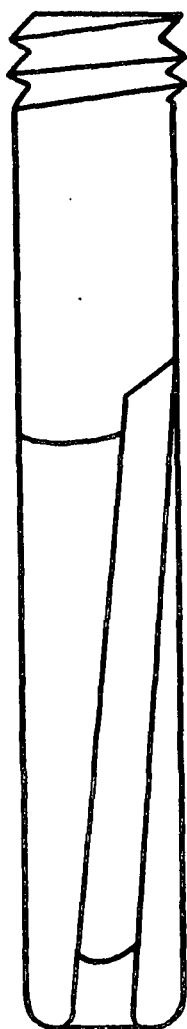


Figure 19. Simple Glass Reactor

solutions to come to temperature. The tube is then inverted six times rapidly to create mixing of the two solutions, and replaced in the thermostat.

At certain time intervals, from 0.5 to 30 hours, the contents of one of these tubes is poured into 4 volumes of 0.5M boric acid to give a resultant pH of 10; this value is obtained immediately. Sodium borohydride is then added to the solution to convert the various sugars to C<sub>6</sub> and C<sub>12</sub> alcohols. This rapid quenching (in less than one minute) in two steps gives a system readily analyzed, as discussed earlier in this report.

This simple glass reactor can be handled manually at lower temperatures, but at higher temperatures (60 and 75°) a system of clamps may be needed for efficient handling. Also, the reaction solution might be quickly transferred by nitrogen pressure into the boric acid solution.

In Fig. 20 is shown a recording from the gas chromatograph of the reaction system obtained from a kinetic run of cellobiose in 2N NaOH for 8.5 hours at 30°C. The large peak is the internal standard, cyclohexyl glucoside. The peak on the right is cellobiose, and the peak at 6 minutes is glucose, formed in the peeling reaction. (Actually, these are the C<sub>12</sub> and C<sub>6</sub> alcohol acetates.) The small peak at about 3 minutes is probably isosaccharinic acid, passing through the gas chromatograph as the acetylated lactone. The very small peak at 5 minutes has not been identified, and may be metasaccharinic acid.

Control experiments were also tried at higher pH values; the results were unsatisfactory. Reduction of cellobiose in 0.5N and 1.5N NaOH (pH 13-14) gave some peeling; this is shown in Fig. 21 where two peaks of low retention time (glucose and glucitol) were obtained. It is interesting that even though sodium

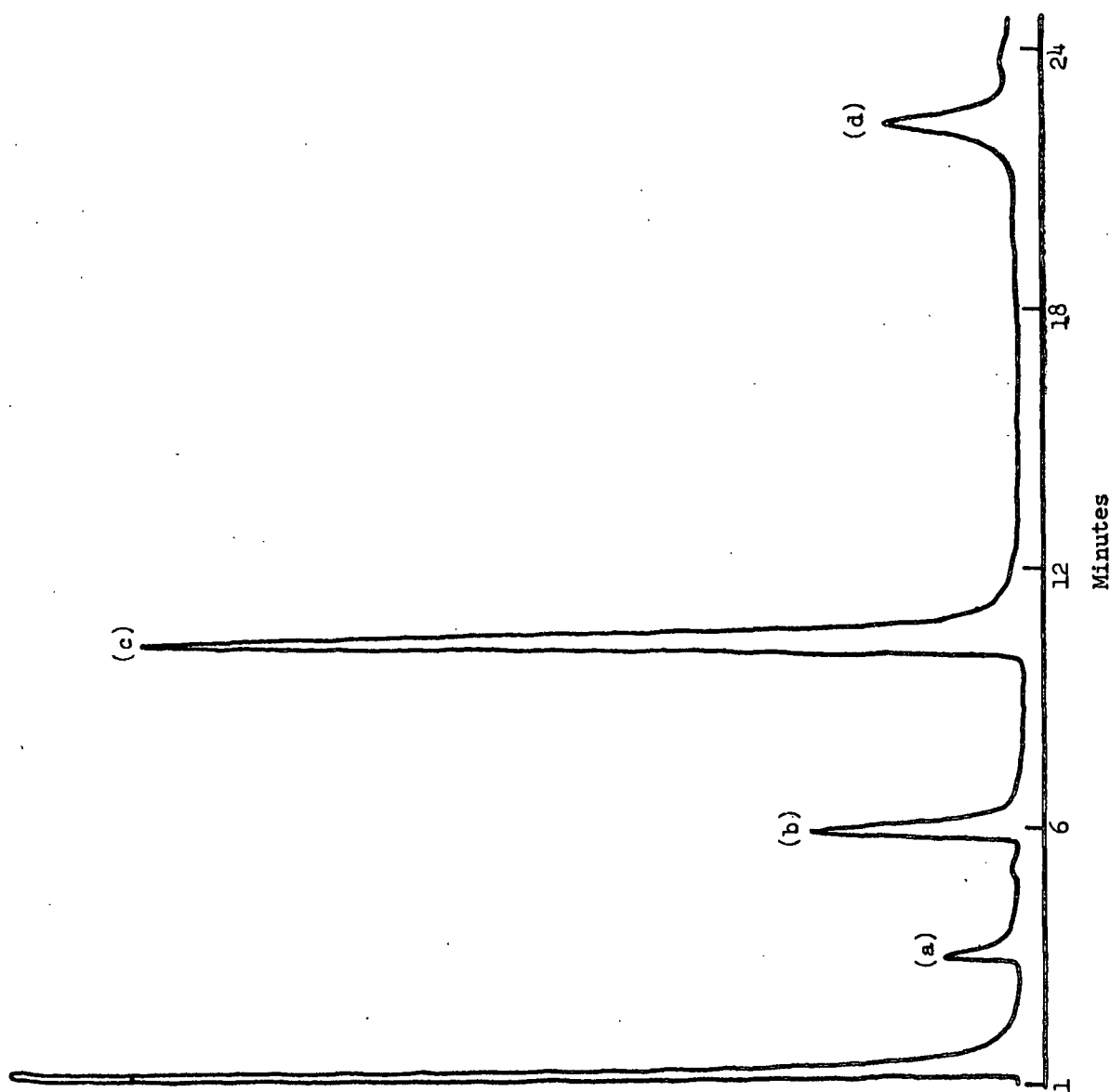


Figure 20. Reaction of Cellobiose with 2N Sodium Hydroxide for 8.5 Hours at 30°C.; (a) Saccharinic Acid, (b) Glucitol, (c) Cyclohexyl Glucoside, (d) Cellobiitol

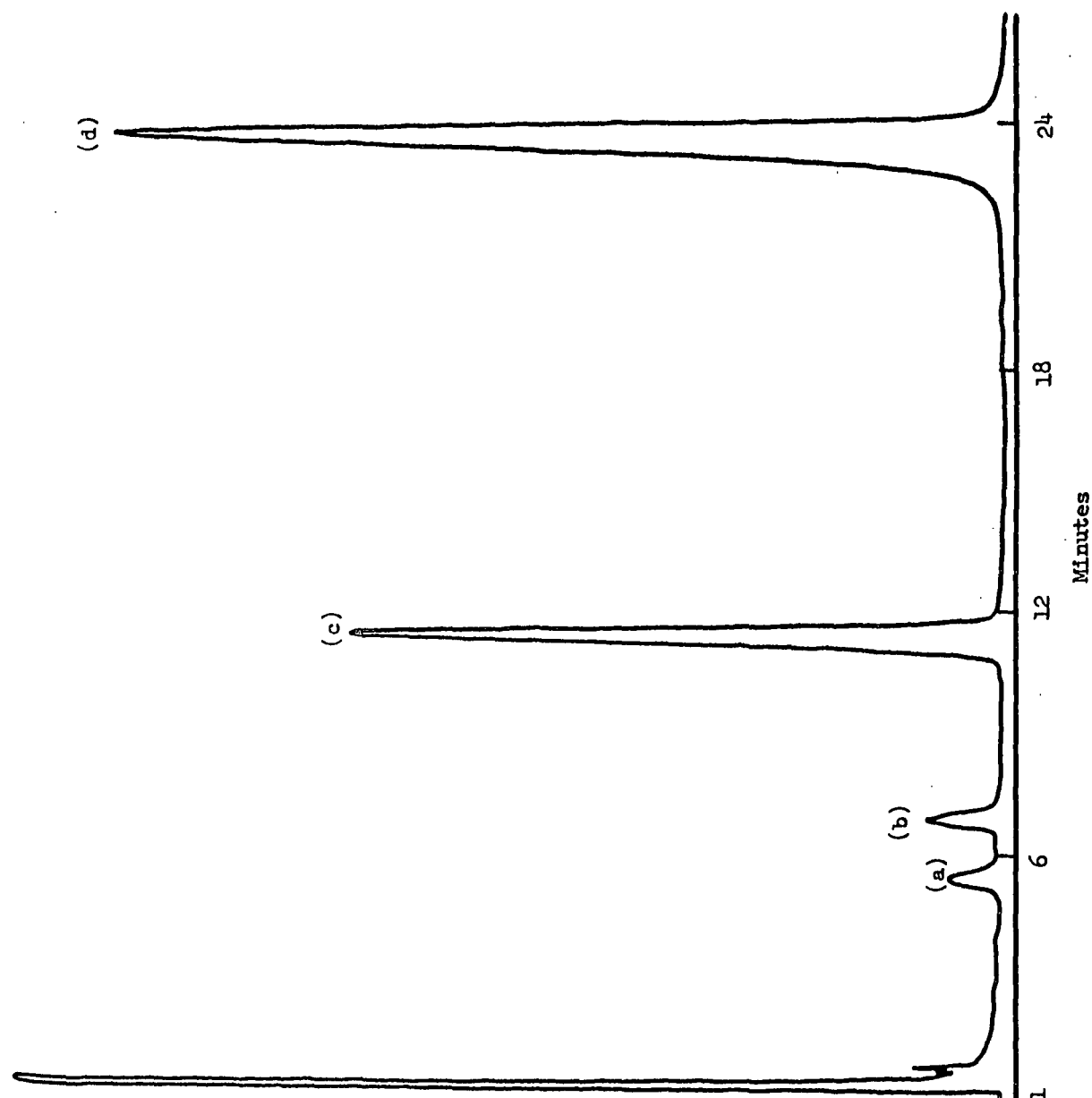


Figure 21. Occurrence of Peeling During Borohydride Reduction of Cellobiose in 1.5N Sodium Hydroxide; (a) Glucose, (b) Glucitol, (c) Cyclohexyl Glucoside, (d) Cellobiitol

borohydride is more stable at pH 13 than at pH 10, the reduction of glucose is incomplete. Apparently glucose, at this higher pH not only undergoes ring opening, but also forms an anion; the latter, with a negative charge, will react slowly with the negatively charged borohydride anion.

This peeling during borohydride reduction at pH 13 makes it impractical, apparently, to quench an alkaline system by adding sodium borohydride directly, without reducing the pH first.

Finally, it should be pointed out that other methods of quenching have been investigated, but the boric acid method seems to be the best. Addition of a cation-exchange resin (IR-120) has been tried; the reduction in pH is slow, taking about 30 sec. MacLaurin and Green (2) added their samples to hydrochloric acid to give a pH of about 2; this method would not be satisfactory for kinetic runs at higher temperatures, as hydrolysis of glycosidic bonds might occur. The 0.5M boric acid has a pH of about 3.8, and even under adverse conditions of mixing during quenching of hot solutions, little hydrolysis should occur.

#### QUENCHING OF ALKALINE SYSTEMS

We have found that a two-stage quenching, as cited earlier, seems to be satisfactory for reactions carried out at lower temperatures, and hope that it can be applied to higher temperatures. The first step, the change of pH, is the important one. The addition of the alkaline solution to boric acid, with stirring, gives a rapid change of pH, from 13 to 10; this change is instantaneous, judging from a visual observation of a pH meter. This lowering of the pH, of course, will slow down the peeling reaction tremendously. The second step, reduction with sodium borohydride, is slower, but is not so important as a time factor. It does

produce sugar alcohols, that are much more resistant to peeling at pH 10 than might occur with the original sugars at this relatively low pH value. Therefore, after this quenching in two steps, the products can be subjected to a leisurely analysis when desired. These two quenching steps should both be carried out within a pressurized system, for reactions above 100°C., as it may take some time to open up such a system and remove samples for analysis.

We have found that a pH of 10 is the best region for the borohydride reduction of sugars. Lindberg, et al. (3) quenched their reactions to pH 9, and then added borohydride. However, in our work at pH 9 the borohydride (sodium hydroxide and boric acid) decomposed fairly rapidly, and a test run with glucose gave only 80% reduction to glucitol. This is shown in Fig. 22; the large and small peaks are glucitol acetate and glucose acetate. In contrast, reduction in a buffered system\* at pH 10 (sodium hydroxide and boric acid) complete reduction was obtained, and gas chromatography showed only one peak, for glucitol acetate.

It should be noted that pH 10 is about the pH of a 1% solution of sodium borohydride and thus reductions of sugars, in such an unbuffered solution go quite readily.

#### FASTER REACTIONS AT ATMOSPHERIC PRESSURE

Two simple flow reactors are shown in Fig. 23. The first one consists of two syringes, containing alkali and sugar solutions, which are forced through a reaction zone Z into a quenching solution C. The length of the tube at Z

\*A buffered system at pH 10 can be obtained by addition of 1 equivalent (100 ml.) of 0.5M boric acid to 1 equivalent (25 ml.) of 2N sodium hydroxide. Similarly, a system at pH 9 can be obtained by use of 2 equivalents (200 ml.) of 0.5M boric acid.

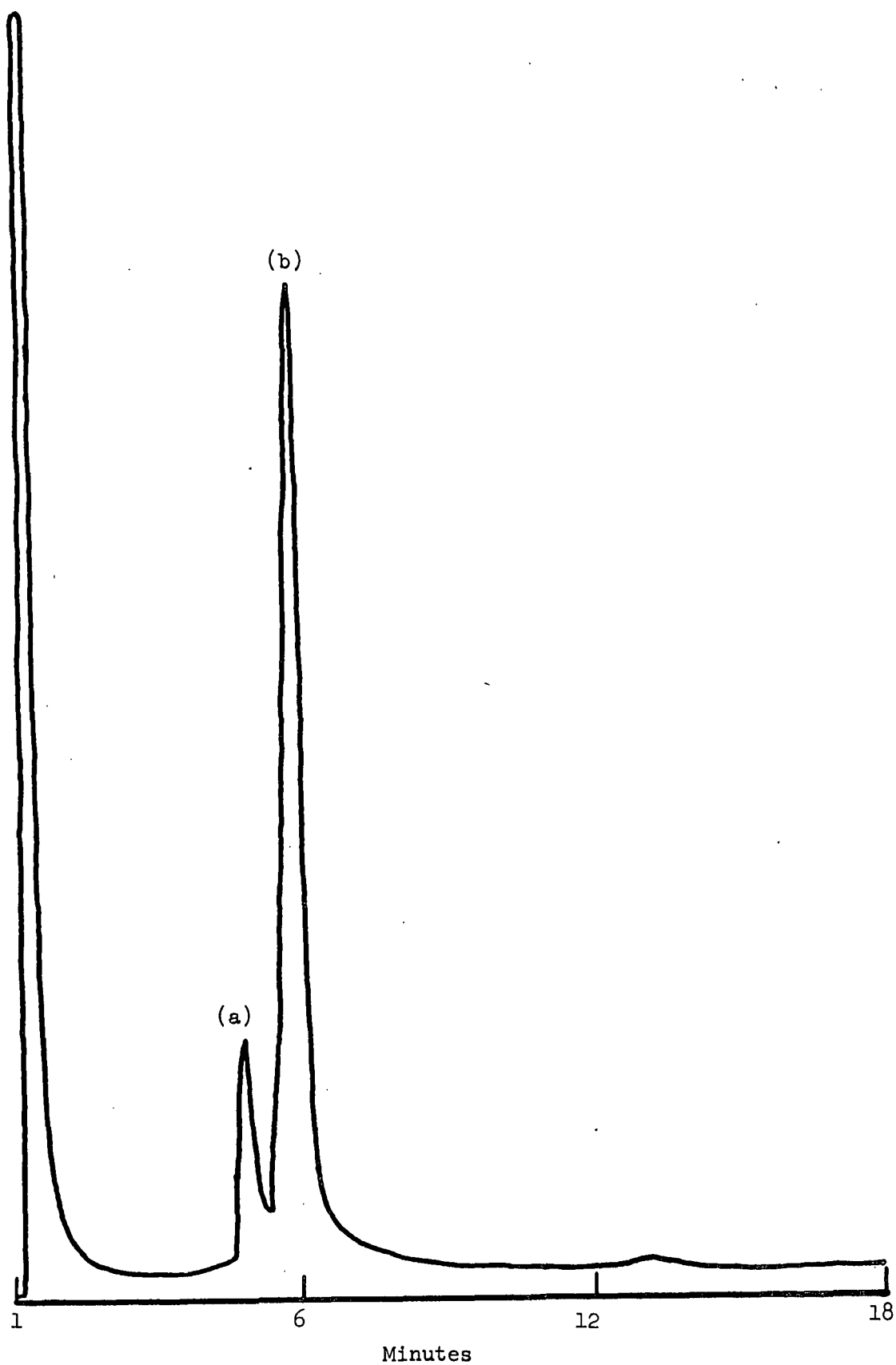


Figure 22. Incomplete Borohydride Reduction of  
Glucose at pH 9, (a) Glucose, (b)  
Glucitol

determines the time of reaction, and this reactor is essentially that of Barman and Gutfreund, mentioned earlier. However, a means of heating the solutions before mixing and during passage through zone Z will have to be devised. The quenching solution Z would be boric acid and would be sufficient to react with all the alkali in syringe A originally. A pushing device above A and B would determine the rate of mixing and flow through the reactor.

The reactor in Fig. 23B is similar, except that the quenching solution is added at a definite rate to the reaction solution after it emerges from the reaction zone Z. Vessel D can be just an empty vessel to collect the quenched solution, or it can contain sodium borohydride solution to carry out the second stage of quenching. In this reactor Solution C would be unheated.

The heating of the reactants in these two devices may be carried out in several ways. Solution A only can be heated directly, and Solution B heated only by virtue of mixing with A. Solution A can be heated directly in the syringe, or heated as it flows out of the syringe toward the mixing zone. It is felt that perhaps it would be best not to heat the sugar solution before mixing, as even at pH 7 some thermal degradation or hydrolysis might occur; such reactions will be even more apparent at higher temperatures.

These two reactors allow only one sample, for a given time of reaction, to be taken. In contrast, the reactor shown in Fig. 24 would allow, with several valves, the taking of several samples after passage through reaction zones of different lengths. Turbulent flow may be an important factor here.

Another reactor which might be feasible for taking several reaction samples is that of Miettinen (9) (see Fig. 6 given earlier in the literature review).



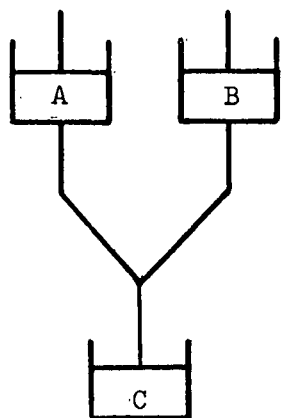


Figure 23A.  
Postulated Flow Reactor

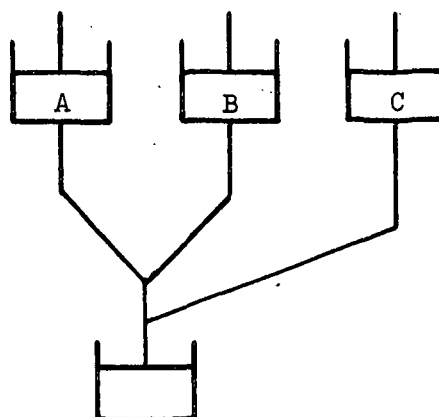


Figure 23B.  
Postulated Flow Reactor

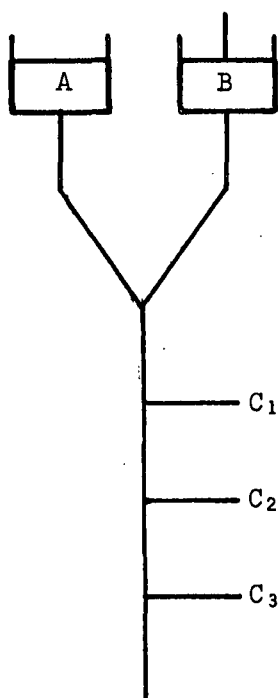


Figure 24. Postulated Flow Reactor for  
Multiple Sampling

The rotating collector is operated with a batch process; however, the advantage of flow reactors shown in Fig. 18 and 19 are that they are a good introduction to the design of equipment to be operated at faster reaction times and under pressure.

The Miettinen apparatus does not possess this advantage.

#### VERY FAST REACTIONS UNDER PRESSURE

Pneumatic reactors, such as those of Chance and Legallis or of Czerlinski (Fig. 16 and 15) operate under pressure. However, the pressure is used primarily as a very fast driving force for the syringes, and apparently all parts of the equipment are at the same temperature. We will be involved with two stages of pressure (a) that of 10 atm. to allow heating aqueous solutions to 170°C., and (b) an additional pressure to drive the solutions through the reactor. Czerlinski speaks of using 50-atm. driving pressure, and Chance and Legallis' apparatus has syringes made of stainless steel and has been tested at "static pressures of 65 atmospheres."

If the pressure packing of such syringes is a difficult problem, it might be possible to put the whole assembly in a pressure vessel, and use external pressure with nitrogen. Then the same pressure would exist within and without the several syringes and connecting lines. Movement of the syringes by a driving platform would have to be controlled by solenoids.

The apparatus of Czerlinski has an open end and pressure is created within the syringes by pneumatic action, to provide fast liquid flow. In contrast, the reactor of Chance and Legallis has a large floating piston just below the observation chamber; this piston moves back to allow collection of the solution displaced from the two syringes containing the reactants. So this reactor has a closed end and it might be feasible to use this type for our work. A third syringe containing

quenching solution should be incorporated within this pressurized system. The combination of syringes, with possibly a fourth syringe for borohydride solution, would be similar to that shown in Fig. 10 or 11 in the Literature Review.

One definite problem is the handling of solutions at two greatly different temperatures. What will their behavior be in a jet mixer? Also, how can we keep a sharp temperature gradient between the two solutions before mixing? Can we have such a gradient and yet a very short interface in the mixing zone? These will all have to be explored.

Dr. Chance informs us that he has explored this area to a certain extent, mixing a relatively large volume at 80-100°C. with a much smaller volume at a lower temperature and did not encounter any difficulty.

## FUTURE WORK

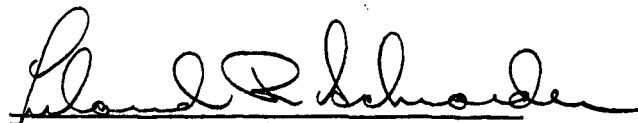
Future work on this project will be divided into two areas. The first and main area will be the obtaining of kinetic data for the peeling reaction in the temperature range of 30 to 70°C. We will attempt to show the relationship of hydroxyl ion concentration to the rate of peeling. Also, we would like to determine the order for reaction rate relative to carbohydrate concentration; presumably this is first-order. The rate constants obtained will be extrapolated (an Arrhenius plot) to give us an idea of the rates to be expected at higher temperatures.

We will explore further the design of flow reactors and try out a simple one at temperatures below 100°C. This will involve mixing of reactants at two different temperatures and quenching the reaction solutions by addition of a suitable solution at lower temperatures. Such experience will be valuable for designing a reactor to be operated under pressure at temperatures above 100°C.

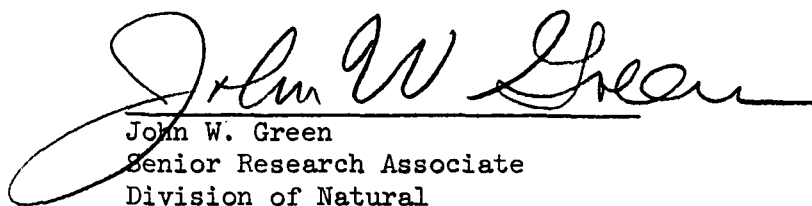
LITERATURE CITED

1. Corbett, W. M., and Kenner, J., J. Chem. Soc. 1955:1431; see also earlier papers in this series, esp. J. Chem. Soc. 1953:2245.
2. MacLaurin, D. J., and Green, J. W., Can. J. Chem. 47:3947, 3957(1969).
3. Lindberg, B., Theander, O., and Uddegard, J., Svensk Papperstid. 69:360 (1966).
4. Borchardt, L. C., and Piper, C. V., Tappi 53:257(1970).
5. Rapid mixing and sampling techniques in biochemistry, Edited by Chance, B., Gibson, Q. H., Eisenhardt, R. H., and Longberg-Holm, K. K., New York, Academic Press, 1964.
6. Hartridge, H., and Roughton, F. J. W., Proc. Royal Soc., London A104:376 (1923).
7. Gibson, Q. H., Discns. Faraday Soc. 17:137(1954).
8. Longberg-Holm, K. K., in Ref. (1), p. 275.
9. Miettinen, J. K., in Ref. (1), p. 306.
10. Barman, T. E., and Gutfreund, H., in Ref. (1), p. 339.
11. Pinsent, B. R. W., Discns. Faraday Soc. 17:140(1954).
12. Chance, B., and Legallis, V., Discn. Faraday Soc. 17:123(1954).
13. Berger, R. L., in Ref. (1), p. 33.
14. Czerlinski, G., in Ref. (1), p. 21.
15. Berger, R. L., in Ref. (1), p. 52.
16. Berger, R. L., and Bowman, R. L., in Ref. (1), p. 57.
17. Chance, B., In Ref. (1), p. 125; see also Chance, B., DeVault, D., Legallis, V., Mela, L., and Yonetani, T. In Fast reactions and primary processes in chemical kinetics. Edited by S. Claesson. Proc. Fifth Nobel Symposium, Aug. 28-Sept. 2, 1967; New York, Interscience, 1967.

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